PCT/IL2004/000692

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APPLICATION NUMBER: ***60/490,259***
FILING DATE: ***July 28, 2003***

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
1) Itamar	WILLNER	Jerusalem, ISRAEL

☑ Additional inventors are being named on the 2nd separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

OPTICAL DETECTION OF DNA, SINGLE BASE MISMATCHES AND TELOMERASE ACTIVITY BY SEMICONDUCTOR NANOPARTICLES

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☐ Customer Number 20529 (Type Customer Number here) → Place Customer Number Bar Code Label here

OR

☑ Firm or Individual Name	NATH & ASSOCIATES PLLC				
Address	1030 15th Street NW, 6th Floor				
Address					
City	Washington	State	DC	ZIP	20005-1503
Country	US	Telephone	202-775-8383	Fax	202-775-8396

ENCLOSED APPLICATION PARTS (check all that apply)

☑ Specification Number of Pages 18
☑ Drawing(s) Number of Sheets 4
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
☑ Other (specify) transmittal letter, postcard

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.
☑ A check or money order is enclosed to cover the filing fees
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☐ Payment by credit card. Form PTO-2038 is attached.

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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Respectfully submitted,

SIGNATURE Todd L. Juneau

TYPED or PRINTED NAME Todd L. Juneau

TELEPHONE 202-775-8383

Date 7/28/03

REGISTRATION NO. (if appropriate) 40,669

Docket Number: 25600

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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PROVISIONAL APPLICATION COVER SHEET
Additional Page

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Docket Number	25600

INVENTOR(S)/APPLICANT(S)

Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
2) Fernando	PATOLSKY	Jerusalem, ISRAEL
3) Yossi	WEIZMANN	Jerusalem, ISRAEL
4) Uri	BANIN	Jerusalem, ISRAEL
5) Ron	GILL	Jerusalem, ISRAEL
6) Taleb	MOKARI	Jerusalem, ISRAEL

Number 2 of 2

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MAIL STOP PROVISIONAL PATENT APPLICATION
Attorney Docket No. 25600

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

WILLNER et al.

Serial No. NOT YET ASSIGNED

Filed: July 28, 2003

For: **OPTICAL DETECTION OF DNA, SINGLE BASE MISMATCHES AND TELOMERASE ACTIVITY BY SEMICONDUCTOR NANOPARTICLES**

TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Submitted herewith for filing in the U.S. Patent and Trademark Office is the following **PROVISIONAL APPLICATION:**

(1) Transmittal Letter;
(2) Cover Sheet for Provisional Application;
(3) 22 page Provisional Application consisting of:
13 pages Textual Specification,
4 pages Claims,
1 page of the Aabstract,
4 sheets Drawings;
(4) Check No. 19030 $ 80.00 for filing fee as a small entity;
(5) Postcard for early notification of serial number.

The Commissioner is hereby authorized to charge any deficiency or credit any excess to Deposit Account No. 14-0112.

Respectfully submitted,
NATH & ASSOCIATES PLLC

By: Todd L. Juneau

Gary M. Nath
Registration No. 26,965
Todd L. Juneau
Registration No. 40,669
Customer No. 20529

Date: July 28, 2003
NATH & ASSOCIATES PLLC
1030th 15TH Street, NW - 6th Floor
Washington, D.C. 20005
GMN/TLJ/dd:APPL.trans

OPTICAL DETECTION OF DNA, SINGLE BASE MISMATCHES AND TELOMERASE ACTIVITY BY SEMICONDUCTOR NANOPARTICLES

FIELD OF THE INVENTION

This invention relates to an analytical method and device for the determination of the presence and the concentration of an analyte in a liquid medium. More specifically, the present invention concerns a fast and sensitive optical method for the detection of cancer, DNA analyte and single base mismatches.

LIST OF REFERENCES

The following references are considered to be pertinent for the purpose of understanding the background of the present invention.

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BACKGROUND OF THE INVENTION

Hybrid systems consisting of semiconductor quantum dots (QDs) coupled to biomaterials find growing interest in the developing research area of nanobiotechnology (1,2). Photochemically-induced fluorescence resonance energy transfer (FRET) between molecular fluorophores (3-5) or the quenching of excited chromophores by metal nanoparticles (6) was reported to probe DNA hybridization processes, and, specifically, the formation and dissociation of hairpin structures. The replication of DNA on bulk surfaces was recently applied for the amplified bioelectronic detection of DNA (7), and the incorporation of redox-active units into the replicated DNA has enabled the electrochemical probing of the dynamics of replication (8). The unique photophysical properties of semiconductor nanoparticles establish the possibility of applying semiconductor nanoparticles as efficient fluorescence labels (9) or as photoelectrochemical probes (10).

SUMMARY OF THE INVENTION

The present invention provides an optical method and device for the determination of an analyte in an assayed sample. The method and device of the invention are based on the use of semiconductor nanoparticles that carry a recognition agent, thus forming a hybrid system. With this system, in the presence of the analyte and under assay conditions, a reaction occurs causing the attachment of an electron acceptor to the recognition agent.

The detection is based on fluorescence resonance energy transfer (FRET) between semiconductor nanoparticle donors, which are excited with electromagnetic radiation, and electron acceptors in the form of dye-labeled agents, preferably dye-labeled nucleic acids. The nanoparticles are excited using a wavelength wherein absorption of the electron acceptor is negligible compared to that of the semiconductor nanoparticle. Efficient resonance energy transfer between the donor semiconductor nanoparticles and the electron acceptors would occur only when the dye-labeled agents are attached to the hybrid system comprising the nanoparticles and the recognition agent. Such attachment may happen only under appropriate reaction conditions and in the presence of the analyte.

The term "*reaction*" is used to denote one or more reactions or interactions carried out at once or in sequence, to attach the electron acceptor, directly or indirectly, to the recognition agent. The "*signal*" in the context of the present invention is emission of light at a given wavelength corresponding to the emission wavelength of the electron acceptor. Accordingly, the term "*assay conditions*" encompasses all the conditions, substances or actions necessary or useful for the appropriate reaction to take place, including sequences of varying conditions or actions. The terms "*attached*" "*attachment*" or "*attaching*" are used to denote binding or other close association between molecules, including covalent bonds, hydrogen bonds, Van der Waals bonds, ionic bonds, etc.

The recognition agent bound to the semiconductor nanoparticles may react with the analyte to form a complex. The complex may then be the attachment site for the electron donor, possibly by a catalyst. Alternatively, the analyte may be a catalyst that can induce a reaction in which the recognition agent is converted into a product. In this alternative, the signal would be present only if the catalyst converted the recognition agent.

The invention permits the qualitative detection of an analyte, namely to get a Yes/No answer whether the analyte exists in the assayed sample, as well as the quantitative detection, namely determine the presence as well as the level of the analyte in the sample. In the following, the term "*determination*" or "*determining*"

or "*detection*" will be used to refer collectively to both qualitative and quantitative assay of the analyte in the assayed sample.

Likewise, the present invention is not limited to the nature of the recognition agent and the analyte, the nature of the reaction or the assay conditions. Nevertheless, it is appreciated that the present invention is especially useful in the determination of DNA or RNA analytes, DNA polymerase or telomerase analytes, cancer cells through telomerase activity and single-base mismatches. In such cases the recognition agent is a single-stranded oligonucleotide.

Thus, according to one aspect of the invention, there is provided a method for determining an analyte in an assayed sample, comprising:

(a) providing semiconductor nanoparticles carrying a recognition agent,

(b) contacting said semiconductor nanoparticles with the assayed sample

(c) providing assay conditions, such that in the presence of the analyte in the assayed sample a reaction would occur, resulting in the attachment of an electron acceptor to the recognition agent,

(d) irradiating the system so as to cause excitation of the semiconductor nanoparticles, transfer of resonance energy from said nanoparticles to the electron acceptor and generation of a signal,

(e) reading the signal.

The semiconductor nanoparticles are preferably nanoparticles. More preferably, the nanoparticles are in the form of quantum dots and even more preferably the nanoparticles are in the form of core-shell layered quantum dots. The signal generated is emission of light.

The excitation of the semiconductor nanoparticles with electromagnetic radiation may be carried out at diverse wavelengths, preferably in the UV or visible

range, depending on the sort of semi-conducting material used and on its form, e.g. particles, nanoparticles, quantum dots, etc.

According to a preferred embodiment, the recognition agent and the analyte form a recognition couple and the detection of the analyte is based on the use of a reagent that binds to the formed couple. A specific example of such embodiment are cases where the analyte is a DNA analyte. In such cases, the assay conditions may comprise DNA polymerase and nucleotide bases, at least one of said nucleotide bases being bound to a dye moiety. Experimental results show that the dye moiety acts as an electron acceptor that absorbs the resonance energy emitted from the semiconductor nanoparticles upon irradiation of said nanoparticles with electromagnetic radiation, and emits light at a different wavelength.

The method of the invention may also provide the determination of at least one base mismatch. For example, the mutant sequence may have a single known base (the mutant base) replacing another base (being any one of the other three nucleotides) in a known position of the normal gene sequence. The recognition agent is a nucleic acid that is shorter than the mutant sequence and the normal gene sequence, and is complementary to both of them up to one base prior to the mutation site, where said recognition agent terminates. Thus, hybridization of nanoparticle bound recognition agent with of any one of said DNAs leaves a single strand portion of said DNAs, beginning at said known position of the mutation.

The assay conditions in this example comprise a DNA polymerase and a dye-bound nucleotide that is complementary to the known base of the mutant sequence. Accordingly, the dye label would bind, through the DNA complex, to the nanoparticles only in complexes comprising the mutant sequence. Subsequently, irradiation of said nanoparticles with electromagnetic radiation would produce the dye specific signal only in case where the mutant analyte was present in the assayed sample This labeling may be intensified by application of thermal dissociation/annealing/labeling cycles, as part of the assay conditions.

A method for the identification of a single base mismatch DNA analyte according to the present invention comprises the following steps:

(a) providing semiconductor nanoparticles carrying a DNA recognition agent that consists of a nucleic acid that is complementary to the DNA analyte up to one base prior to the mutation site,

(b) contacting said semiconductor nanoparticles with the assayed sample,

(c) providing assay conditions that give rise to a reaction, where said assay conditions comprise DNA polymerase and a nucleotide base bound to a dye molecule that acts as an electron acceptor, wherein the nucleotide base is complementary to the base of the mutation,

(d) irradiating the system so as to cause excitation of the semiconductor nanoparticles, transfer of resonance energy from said nanoparticles to said electron acceptor and generation of a signal, and

(e) reading said signal.

The method of the invention requires that the nanoparticles be excited in a region of wavelengths where absorption of the dye is negligible compared to that of the nanoparticles and under conditions where the emission of the nanoparticles overlap with the absorbance of the dye. A non-limiting example of a dye used in the method of the invention is Texas Red.

According to another embodiment, the analyte to be detected is a catalyst that can induce a reaction in which the recognition agent is converted into a product. A specific example of catalysts are enzymes, e.g. telomerase. The detection of telomerase activity is indicative of the presence of cancer cells. A method for detecting cancer cells according to the present invention comprises:

(a) providing semiconductor nanoparticles carrying a single-stranded DNA recognition agent, that serves as a primer for telomerase reaction,

(b) providing an assay sample comprising cellular extract from one or more cells suspected of being cancerous;

(c) contacting said semiconductor nanoparticles with the assayed sample

(d) providing assay conditions that give rise to a reaction; wherein said assay conditions comprise nucleotide bases and at least one of said nucleotide bases being bound to an electron acceptor,

(e) irradiating the system so as to cause excitation of the semiconductor nanoparticles, transfer of resonance energy from said nanoparticles to said electron acceptor and generation of a signal, and

(f) reading said signal.

According to another aspect, the present invention provides a bio-sensing device for determining an analyte in an assayed sample, the device comprising:

(One) an assay cell comprising a system of semiconductor nanoparticles carrying recognition agents such that in the presence of an analyte and under assay conditions a reaction occurs and an electron acceptor comprised in said assay conditions, is bound to said system,

(Two) irradiation unit for exciting said semiconductor nanoparticles, such that in the presence of an electron acceptor bound to said system as a consequence of said reaction that took place in step ((One) above, a signal is generated, and

(Three) measuring utility for reading the signal.

In another aspect, the present invention provides a bio-sensing system for determining the presence of two or more different analytes in an assayed sample, the system comprising:

(a1) a plurality of assay cells, each cell for determining a specific analyte of the two or more different analytes, and having at least one cell for each of said different analytes, each of said cells comprising a system of semiconductor nanoparticles carrying recognition agents such that in the presence of the specific analyte and under assay conditions, a reaction occurs and an electron acceptor comprised in said assay conditions, is specifically bound to said system,

(b1) irradiation unit for exciting said semiconductor nanoparticles, such that in the presence of an electron acceptor bound to said system, a signal is generated, and

(c1) detecting utility for detecting the signal.

The detecting utility in the above system is such that may identify which of the devices in the system gave rise to the signal (and accordingly assist in the identification of the analyte(s) in the assayed sample) or measure the intensity of the signal, or both.

The above system, comprising an array of bio-sensing devices is especially useful as a DNA array detection. The separate bio-sensing devices are bound to a substrate by any surface modification technique. Thus, for example, if the substrate is made of gold or glass, the nanoparticles may be attached thereto through di-thiol linkers. The signals generated by the system may be read out by surface imaging techniques, where the emission of photons from areas bound to an analyte, is different from the emission from areas which did not bind an analyte.

Examples of semiconducting materials to be used in the device of the invention are Group III-V, Group III-V alloys, Group II-VI, Group I-VII, Group IV and alloys thereof. More specific examples of semiconducting materials are InAs, GaAs, GaP, GaSb, InP, InSb, AlAs, AlP, AlSb, InGaAs, GaAsP, InAsP, CdS, CdSe, CdTe, ZnS, ZnSe, ZnTe, HgS, HgSe, HgTe, CuCl, CuBr, CuI, AgCl, AgBr, AgI, Si, Ge and alloys thereof. The nanoparticles are preferably nanoparticles having the

shape of quantum dots. More preferably the nanoparticles are in the form of core-shell layered quantum dots.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 is a scheme, which illustrates the telomerization and replication processes on CdSe-ZnS quantum dots functionalized with nucleic acids and with the incorporation of Texas Red-labeled dUTP.

Fig. 2A shows the emission spectra upon the time-dependent telomerization on the CdSe-ZnS QDs: (i) - before addition of telomerase, (ii), (iii) and (iv): after 10, 30 and 60 minutes of telomerization, respectively.

Fig. 2B shows the AFM image of CdSe-ZnS QDs before telomerase treatment.

Fig. 2C shows the AFM image of a CdSe-ZnS QD after 60 minutes telomerization (images in Figures 2A and 2B are recorded on mica surfaces activated with 5mM MgCl2).

Fig. 3A shows the emission spectra upon the time-dependent DNA-Replication on the CdSe-ZnS QDs: (i)- before addition of dye-dUTP, (ii), (iii) and (iv): after 1, 30 and 60 minutes of replication, respectively.

Fig. 3B shows the AFM image of CdSe-ZnS quantum dots hybridized with M13ϕ DNA.

DETAILED DESCRIPTION OF THE INVENTION

The method of the present invention is schematically illustrated in **Fig. 1**. CdSe-ZnS biocompatible quantum dots (hereinafter abbreviated "*QDs*") were used as photochemical centers for telomerization or DNA replication occurring on the particles of a telomerase-recognized nucleic acid or a DNA (or RNA) hybridized to

a nucleic acid associated to a quantum dot in the presence of a telomerase or a polymerase or reverse transcriptase in the presence of all nucleotide mixture that includes a dye-labeled nucleotide such as dye-labeled dUTP or dCTP. . The dye units incorporated into the new synthesized telomer or DNA replica enable fluorescence energy transfer (FRET) from the excited particles to the dye and the observation of the fluorescence characteristic to the dye.For the telomerase analysis, according to **Fig. 1**, CdSe-ZnS nanoparticles (4.2 nm diameter, λ_{em}= 400 nm, 20% luminescence quantum yield) stabilized by the mercaptopropionic ligand were modified with the thiolated oligonucleotide (**1**) (sequence: 5'-HS-$(CH_2)_6$-TTTTTTAATCCGTCGAGCAGAGTT-3'). Analysis of the nanoparticles indicated that ca. 25 nucleic acid units were associated with each nanoparticle. The (**1**)-functionalized semiconductor QDs were incubated with a dNTP mixture (dATP, nucleotide Texas-Red 14-dUTP, (**2**)), (100 μM) in the presence of telomerase (extracted from HeLa cells, 10.000 cells). **Fig. 2A** shows the fluorescence spectra of the system upon excitation of the CdSe-ZnS QDs at λ= 400 nm, as a function of telomerization time. The CdSe-ZnS QDs prior to the introduction of telomerase emitted at λ=560 nm. After the addition of telomerase, and as telomerization proceeds, the fluorescence of the QDs decreased, with the concomitant increase of the characteristic emission of the dye at λ=610 nm.

Control experiments revealed that excitation of the nucleotide mixture that includes the dye modified dUTP in the absence of the CdSe-ZnS QDs at λ= 400 nm does not lead to an observable emission at λ=560 nm. However, excitation of the system at λ=550 nm lead to a high fluorescence signal characteristic to the dye. As the emission of the CdSe-ZnS QDs at λmax=560 nm coincides with the absorbency band of the dye (**2**), the emission observed upon telomerization is attributed to fluorescence resonance energy transfer (FRET) from the nanoparticles to the dye molecules incorporated into the telomeric units by telomerase.

Atomic Force Microscopy (AFM) images of the CdSe-ZnS QDs prior to the telomerization, **Fig. 2B**, and the image of a nanoparticle after telomerization, **Fig.**

2C were recorded on freshly cleaved mica surfaces, under ambient conditions. While the height of the nanoparticle is ca. 4 nm, its lateral dimensions are distorted due to the tip dimensions. The nanoparticle after telomerase incubation reveals beautifully the synthesized hinged DNA strands. The height of the telomeric DNA chains is ca. 1 nm, whereas their length is ca. 300 nm. This corresponds to a telomerase induced elongation of ca. 1000 base units in the telomeric DNA chain.

The CdSe-ZnS quantum dots enabled also the probing of the dynamics of polymerase replication of DNA, as showed schematically in Fig. 1. In this system the primer **(3)** (sequence: '5-HS-$(CH_2)_6$-CCCCCACGTTGTAAAACGACGG-CCAGT-3') complementary to M13ϕDNA was assembled on the CdSe-ZnS QDs. Hybridization of the **(3)**-functionalized QDs with M13ϕ DNA followed by replication in the presence of polymerase (Klenow fragment, 10 units) and a mixture of dNTPs containing the fluorophore-labeled nucleotide, yield the dye-labeled DNA replica. The dynamics of DNA replication could be followed by FRET from the luminescent QDs to the incorporated dye unit, as shown in **Fig. 3A**. An AFM image of two CdSe-ZnS nanoparticles hybridized to M13ϕ DNA is shown in **Fig. 3B**.

The method and device of the invention may be applied for the fast and sensitive detection of cancer cells and as an amplification route for analyzing DNA on chip arrays. The polymerase-induced replication of DNA on the surface of luminescent QDs represents an "AND" gate where FRET readout occurs only if hybridization and replication proceed.

Synthesis of functionalized nanoparticles:

Synthesis of CdSe-ZnS nanoparticles: trioctylphospine oxide (TOPO) protected nanoparticles, soluble in organic solvents were reacted in toluene (1ml) with 100μl of mercaptopropionic acid overnight. (Protected from light with aluminum foil). After this incubation time the nanoparticles were transferred to water by adding 1M KOH solution (1ml), the two phase mixture was separated and this process repeated a second time with clean toluene (1ml). The water-soluble

CdSe-ZnS nano-particles were separated from the excess of mercaptopropionic acid by 3 repeated cycles of precipitation of the nanoparticles with acetone and followed by the re-dissolution of the nanoparticles in a phosphate buffer solution pH 7.4, 10mM.

Preparation of DNA-modified CdSe-ZnS nanoparticles: The mercaptopropionic acid functionlized CdSe-ZnS nanoparticles (0.1 O.D) were reacted with freshly reduced and purified thiolated oligonucleotides ((1) or (3)) 6-10 O.D) overnight in order to allow the exchange of the thiol group associated with the nanoparticles with thiolated oligonucleotide. The DNA modified nanoparticles were separated from the free oligonucleotides by the use of micro-spin filter (Millipore, 30KDa) or by acetone precipitation and re-dissolution in phosphate buffer solution. The nucleic acid-modified nanoparticles were obtained with a 70% yield. The fluorescence quantum yield of the nucleic acid-functionalized nanoparticles is ca. 15%.

The analysis of M13ϕ DNA by FRET: 0.1 O.D of DNA (3)-modified CdSe-ZnS nanoparticles were incubated in a solution containing a mixture of dATP, dCTP, dGTP (0.5mM each) and Texas-Red 14-dUTP (100μM) in the presence of M13ϕ DNA, 1nM concentration. The luminescence of the sample was followed in a quartz cuvette at different time intervals of replication λexcitation=400nm.

The analysis of telomerase activity by FRET: 0.1 O.D of the (1)-modified CdSe-ZnS nanoparticles was incubated in a solution containing a mixture of dATP, dCTP, dGTP (0.5mM each) and Texas-Red 14-dUTP (100μM) in the presence of telomerase, (cell extracts containing telomerase in 1xChaps buffer). The luminescence of the sample was followed in a quartz cuvette at different time intervals of replication λexcitation=400nm.

Imaging the telomerization on the CdSe-ZnS nanoparticles by AFM: Freshly-cleaved Ruby-mica surfaces were used (activated with 5mM $MgCl_2$). A drop of

nano-particles solution (before or after telomerization) was placed on the mica surface and after evaporation the surface was wash with 2x100μl water, dried under a gentle flow of argon and the AFM were recorded.

CLAIMS:

1. A method for determining an analyte in an assayed sample, comprising:

(a) providing semiconductor nanoparticles carrying a recognition agent,

(b) contacting said semiconductor nanoparticles with the assayed sample

(c) providing assay conditions, such that in the presence of the analyte in the assayed sample a reaction would occur, resulting in the attachment of an electron acceptor to the recognition agent,

(d) irradiating the system so as to excite the semiconductor nanoparticles, whereby resonance energy is transferred from said nanoparticles to the electron acceptor and an electromagnetic signal is generated, and

(e) reading the signal.

2. The method of claim 1 wherein said nanoparticles are in the form of quantum dots.

3. The method of claim 1, wherein said signal is emission of light.

4. The method of anyone of claims 1 to 3 wherein the recognition agent and the analyte form a recognition couple and the detection of the analyte is based on the use of a reagent that binds to the formed pair.

5. The method of claim 4, wherein said analyte is a DNA analyte.

6. The method of claim 5, wherein the assay conditions comprise DNA polymerase and nucleotide bases, at least one of said nucleotide bases being bound to a dye moiety.

7. The method of according to anyone of claims 1 to 6, wherein said dye acts as an electron acceptor that absorbs the resonance energy emitted by the semiconductor nanoparticles upon irradiation of said nanoparticles with electromagnetic radiation.

8. The method of anyone of Claims 1 to 7, wherein said nanoparticles are excited in a region where absorption of the dye is negligible compared to that of the nanoparticles.

9. The method of anyone of Claims 1 to 7, wherein said dye molecule is a fluorescent material.

10. The method of claim 5, wherein the analyte is a nucleotide sequence having at least one base mismatch.

11. The method according to Claim 10, wherein the assay conditions comprise DNA polymerase and a nucleotide base complementary to the single base mismach and being bound to a dye moiety.

12. The method of claim 1 wherein the analyte is a catalyst that can induce a reaction in which the recognition agent is converted into a product.

13. The method of claim 12, wherein the catalyst is an enzyme.

14. The method of claim 13, wherein the enzyme is telomerase.

15. The method of claim 14, wherein the assayed sample comprises cellular extract.

16. The method of claim 14 for the detection of cancer cells.

17. The method of claim 16 comprising:

(a) providing semiconductor nanoparticles carrying a single-stranded DNA recognition agent, that serves as a primer for telomerase reaction,

(b) providing an assay sample comprising cellular extract from one or more cells suspected of being cancerous;

(c) contacting said semiconductor nanoparticles with the assayed sample

(d) providing assay conditions that give rise to a reaction; wherein said assay conditions comprise nucleotide bases and at least one of said nucleotide bases being bound to an electron acceptor molecule;

(a) irradiating the system so as to cause excitation of the semiconductor nanoparticles, transfer of resonance energy from said nanoparticles to said electron acceptor and generation of a signal, and

(f) reading said signal.

18. A bio-sensing device for determining a specific analyte in an assayed sample, the device comprising:

(a) an assay cell comprising a system of semiconductor nanoparticles carrying recognition agents such that in the presence of the specific analyte and under assay conditions a reaction occurs,

(b) irradiation unit for exciting said semiconductor nanoparticles, such that in the presence of an electron acceptor bound to said system as a consequence of the reaction that took place in step ((a) above, a signal is generated, and

(c) measuring utility for reading the signal.

19. The device of claim 18, wherein said nanoparticles are in the form of quantum dots.

20. The device of claim 18 wherein the excitation of the semiconductor nanoparticles is by electromagnetic radiation.

21. The device of claim 20 wherein the electromagnetic radiation is in the UV or visible range.

22. The device of claim 18 wherein the semi-conducting material is selected from: Group III-V, Group III-V alloys, Group II-VI, Group I-VII, and Group IV semiconductors.

23. The device of claim 22 wherein the semi-conducting material is selected from InAs, GaAs, GaP, GaSb, InP, InSb, AlAs, AlP, AlSb, InGaAs, GaAsP, InAsP, CdS, CdSe, CdTe, ZnS, ZnSe, ZnTe, HgS, HgSe, HgTe, CuCl, CuBr, CuI, AgCl, AgBr, AgI, Si, Ge and alloys thereof.

24. The device of claim 19 wherein said nanoparticles are in the form of core-shell layered quantum dots.

25. The method of claim 1 wherein said nanoparticles are in the form of core-shell layered quantum dots.

26. A bio-sensing system for determining the presence of two or more different analytes in an assayed sample, the system comprising:

(a1) a plurality of assay cells, each cell for determining a specific analyte of the two or more different analytes, and having at least one cell for each of said different analytes, each of said cells comprising a system of semiconductor nanoparticles carrying recognition agents such that in the presence of the specific analyte and under assay conditions, a reaction occurs and an electron acceptor comprised in said assay conditions, is specifically bound to said system,

(b1) irradiation unit for exciting said semiconductor nanoparticles, such that in the presence of said electron acceptor bound to said system, a signal is generated, and

(c1) detecting utility for detecting the signal.

ABSTRACT

The present invention provides an optical method and device for the determination of an analyte in an assayed sample. The method and device of the invention are based on the use of semiconductor nanoparticles that carry a recognition agent. The detection is based on fluorescence resonance energy transfer (FRET) between the semiconductor nanoparticle donors, which are excited with electromagnetic radiation, and electron acceptors in the form of dye-labeled agents, that are attached to the recognition agent in the presence of the analyte and under assay conditions. The nanoparticles are excited using a wavelength wherein absorption of the electron acceptor is negligible compared to that of the semiconductor nanoparticle. Furthermore, the emission of the nanoparticles should overlap the absorbance of the electron acceptors.

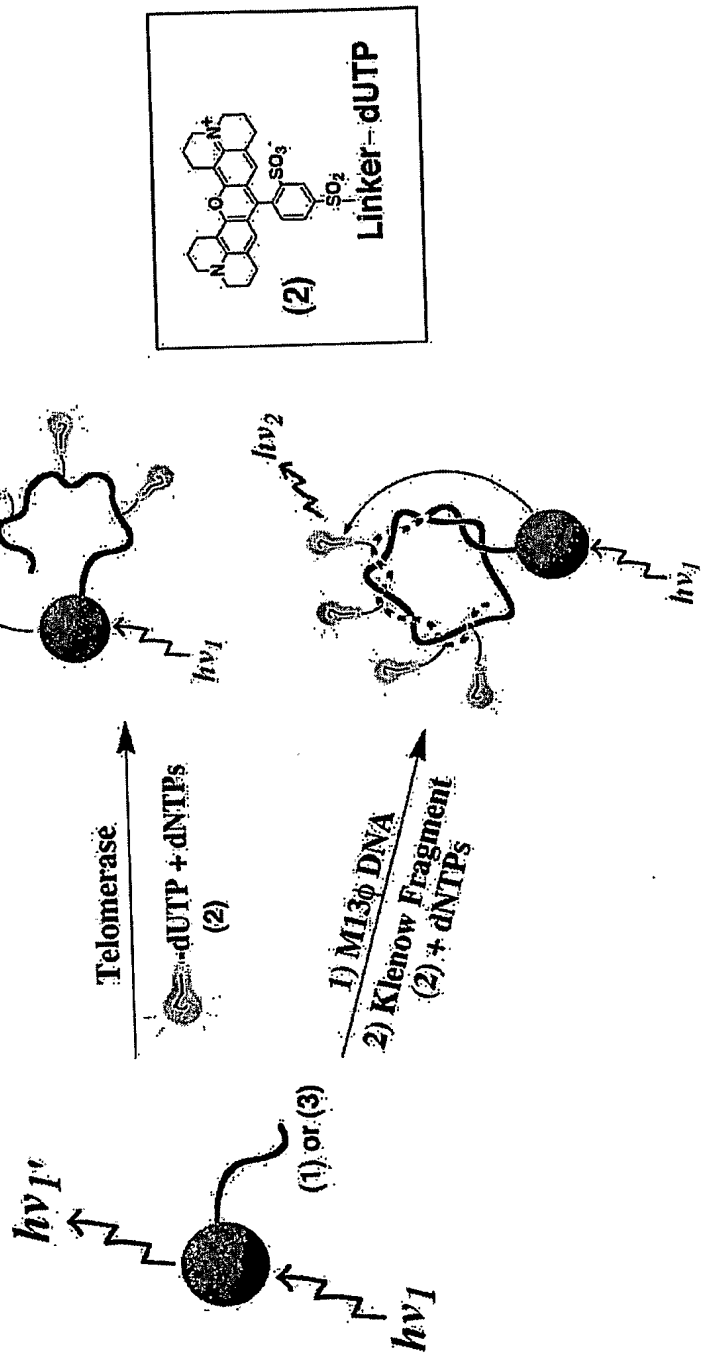

FIG.1

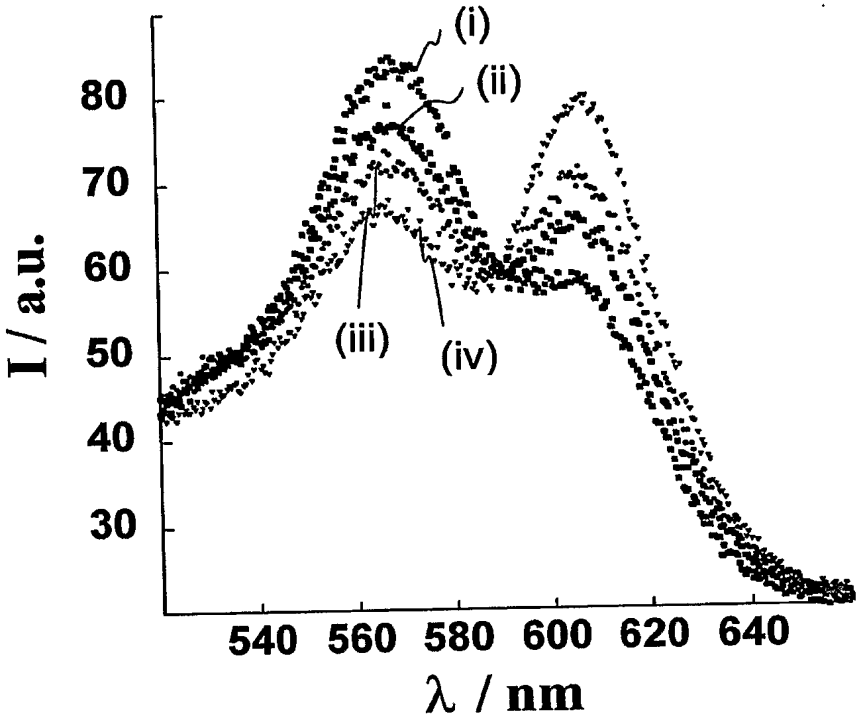

FIG. 2A

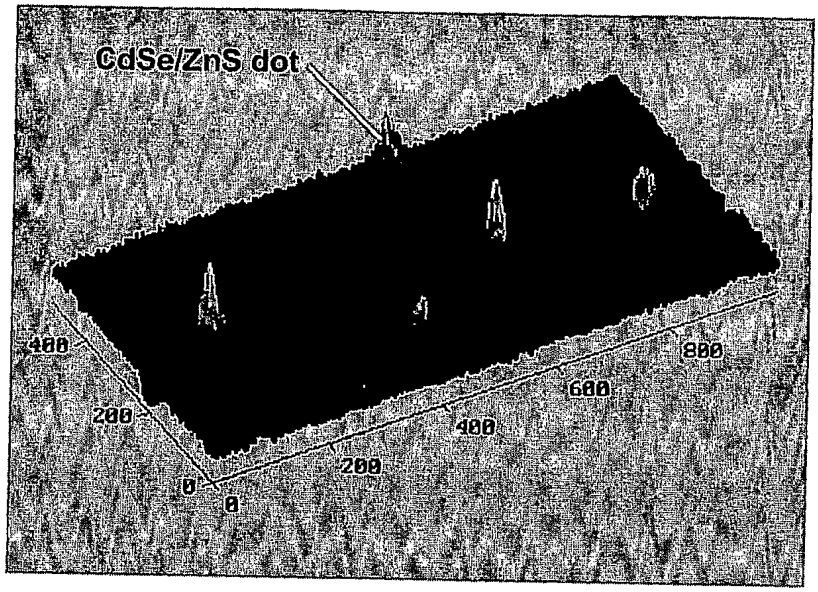

FIG. 2B

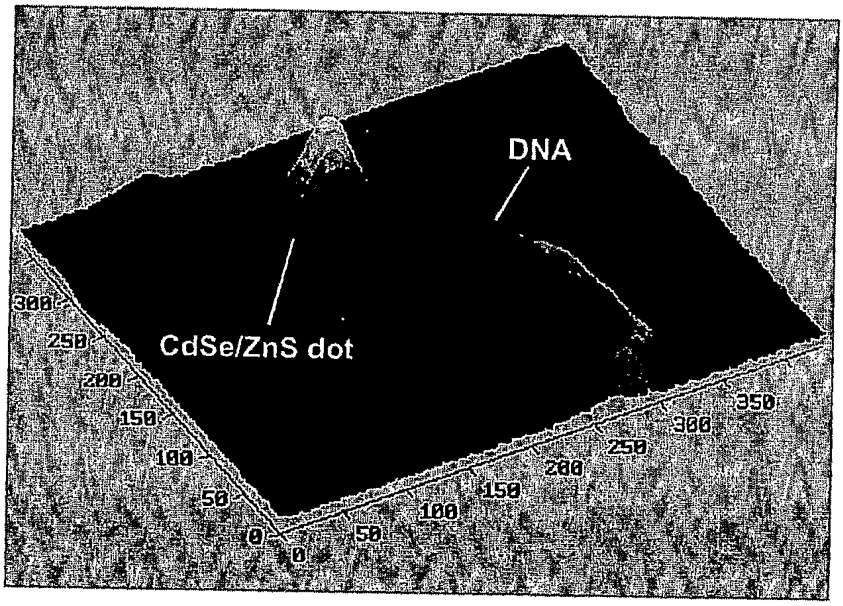

FIG. 2C

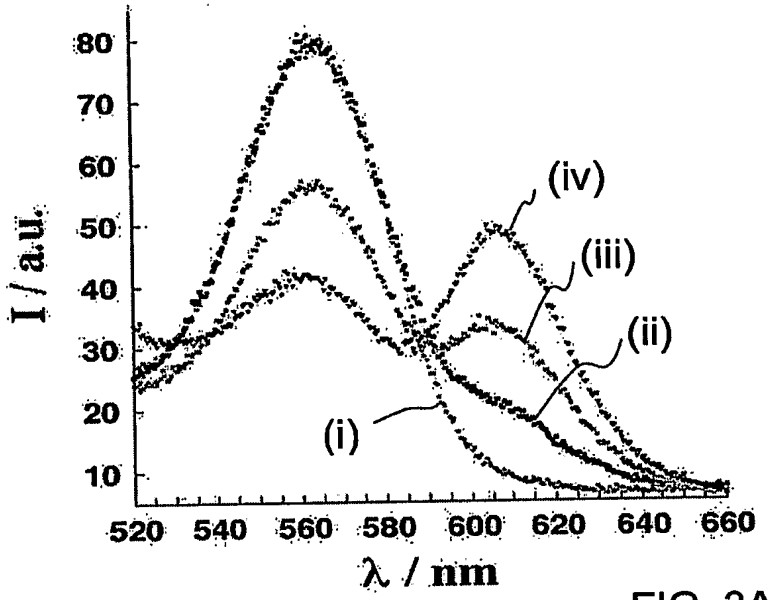

FIG. 3A

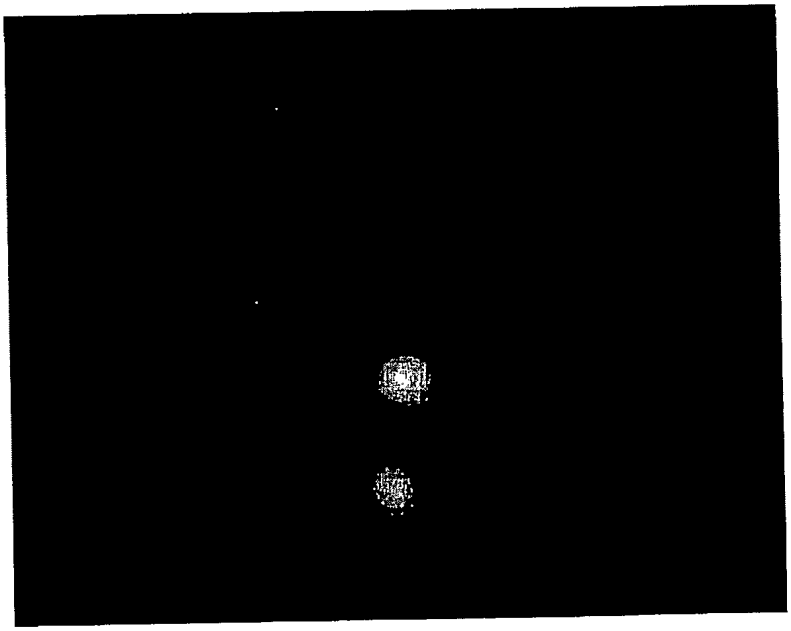

FIG. 3B